May 17, 2004

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Senior Toxicologist
The American Petroleum Institute
Petroleum HPV Testing Group
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Washington, DC 20005-4070

Dear Dr. Gray:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for the Crude Oil Category posted on the ChemRTK HPV Challenge Program Web site on December 19, 2003. I commend The American Petroleum Institute Petroleum HPV Testing Group for its commitment to the HPV Challenge Program.

EPA reviews test plans and robust summaries to determine whether the reported data and test plans will provide the data necessary to adequately characterize each SIDS endpoint. On its Challenge Web site, EPA has provided guidance for determining the adequacy of data and preparing test plans used to prioritize chemicals for further work.

EPA will post this letter and the enclosed comments on the HPV Challenge Web site within the next few days. As noted in the comments, we ask that the HPV Testing Group advise the Agency, within 90 days of this posting on the Web site, of any modifications to its submission. Please send any electronic revisions or comments to the following e-mail addresses: oppt.ncic@epa.gov and chem.rtk@epa.gov.

If you have any questions about this response, please contact Richard Hefter, Chief of the HPV Chemicals Branch, at 202-564-7649. Submit questions about the HPV Challenge Program through the "Contact Us" link on the HPV Challenge Program Web site pages or through the TSCA Assistance Information Service (TSCA Hotline) at (202) 554-1404. The TSCA Hotline can also be reached by e-mail at tsca-hotline@epa.gov.

I thank you for your submission and look forward to your continued participation in the HPV Challenge Program.

Sincerely,

-*S*-

Oscar Hernandez, Director
Risk Assessment Division

Enclosure

cc: W. Penberthy
M. E. Weber

EPA Comments on Chemical RTK HPV Challenge Submission:
Crude Oil Category

Summary of EPA Comments

The sponsor, The American Petroleum Institute, submitted a test plan and robust summaries to EPA for the Crude Oil Category together with a cover letter dated November 24, 2003. EPA posted the submission on the ChemRTK HPV Challenge Web site on December 19, 2003. The category consists of light, medium and heavy crude oils, in addition to tar sands, irrespective of source or hydrocarbon composition, that are covered under one CAS Registry Number, 8002-05-9 (crude petroleum).

EPA has reviewed this submission and has reached the following conclusions:

1. Category Definition. The Crude Oil category contains one CAS Number, 8002-05-9 (crude petroleum), but covers all crude oils regardless of source or hydrocarbon composition as well as synthetic crude oil that is derived from tar sands (see Table 1). These crude oils are a complex mixture of paraffinic, naphthenic and aromatic hydrocarbons ranging in carbon number from C1 to C60+.

2. Category Justification. The toxicity data provided by the submitter appear to support the grouping of crude oils into a single category. However, it is hard to see the correlations among composition, API gravity and the toxicity data as presented in the test plan. For the physicochemical and environmental fate properties, how the values submitted correlate with differences in the compositions of paraffins, naphthenes and aromatic hydrocarbons in the crude oils is not clear. Also, the expectation that the mammalian toxicities of these crude oils will correlate with the PAC content of the crude oils is not supported by the data provided in the test plan. In addition, the proportion of aromatic hydrocarbons in the crude oils does not always correlate with API gravity. Furthermore, from the information provided by the submitter, it is not possible to determine whether volume percent aromatic content can be an accurate indicator of 3-7 ring PAC content, which is considered by the submitter to be the basis of observed mammalian toxicity in the crude oils. For ecological effects, although the studies submitted for fish and aquatic invertebrates are adequate, it is not clear that adequate data exist to characterize the *extremes* of aquatic toxicity of crude oils to aquatic organisms because the substances that have been tested were not adequately characterized with respect to components that could affect their toxicity.

Therefore, the submitter needs to clarify how the members of the crude oils category are related by a similar carbon number range whose physicochemical properties are expected to reflect the proportions of paraffins, naphthenes and aromatic hydrocarbons in the mixtures. Also, the submitter needs to provide information that demonstrates a pattern of increasing toxicity with increasing aromatic content and 3-7 ring PAC content, and how the substances that have been tested for aquatic toxicity represent the members of the crude oils category.

3. Physicochemical Properties and Environmental Fate. The data provided by the submitter for these endpoints are adequate for the purposes of the HPV Challenge Program. EPA recommends that the submitter also provide estimated distributions of the model hydrocarbons based on a Level III fugacity model.

4. Health Effects. EPA agrees with the submitter's proposal to test two samples based on the 3-7 ring PAC content if the submitter can demonstrate that toxicity does vary with PAC content. In addition, the submitter needs to provide mutagenicity data for a crude that has a high PAC content because the data provided are limited in the number of strains tested and reflect metabolic activation tests only. EPA reserves judgment on any additional testing needs (i.e., number and types of tests) pending receipt of more information on the content of metals and any other known or suspected toxicants in crude oils.

5. Ecological Effects. EPA reserves judgment on the fish and aquatic invertebrate toxicity endpoints pending more information on how the substances that have been tested represent the members of the

crude oils category. In addition, EPA disagrees with the submitter that no additional testing for aquatic plants is necessary. The submitter needs to conduct acute toxicity testing on this endpoint according to the OECD guidelines. Also, no chronic aquatic toxicity data were included in the test plan or robust summary document. Because the Log K_{ow} values of some components of the crude oils are >4.2, there appears to be a need for chronic aquatic toxicity testing.

EPA requests that the submitter advise the Agency within 90 days of any modifications to its submission.

EPA Comments on the Crude Oil Category Challenge Submission

Category Definition

The Crude Oil category contains one CAS Number, 8002-05-9 (crude petroleum), but covers all crude oils regardless of source or hydrocarbon composition as well as synthetic crude oil that is derived from tar sands (see Table 1). These crude oils are complex mixtures of paraffinic, naphthenic and aromatic hydrocarbons ranging in carbon number from C1 to C60+. The proportions of paraffinic, naphthenic, aromatic hydrocarbons, and other components vary among geographic regions, and even within a single geologic formation. The crude oils are classified as paraffinic or naphthenic crude oils on the basis of their components. Paraffinic crude oils are rich in linear and branched paraffins, have a high API gravity, low density and viscosity, and contain a higher concentration of gasoline grade naphtha. Naphthenic crude oils contain mainly cycloparaffins and aromatic hydrocarbons, have a low API gravity, higher density and viscosity, and contain other materials (e.g., metals such as nickel, iron, vanadium, and arsenic) and heteroatoms (e.g., sulfur-, nitrogen-, and oxygen-containing hydrocarbon analogs). The submitter further divided the crude oils into light, mid-range, and heavy (specific gravities of <0.82, 0.82-0.97, >0.97, respectively). Paraffinic crude oils are often classified as "light" while aromatic crude oils are often classified as "heavy," although this is not always the case.

Table 1. Examples of Crude Oils Covered Under CAS Number 8002-05-9 (Crude Petroleum)[a]

Crude Oil Source	Paraffins (% vol)	Naphthenes (% vol)	Aromatics (% vol)	Sulfur (% wt.)	API gravity (^{0}API)
Light Crude Oils					
Saudi Light	63	18	19	2.0	34
South Louisiana	79	45	19	0.0	35
Beryl	47	34	19	0.4	37
North Sea Brent	50	34	16	0.4	37
Nigerian Light	37	54	9	0.1	36
Lost Hills Light	Non-aromatics 50%		50	0.9	-
USA Mid-Continent sweet	-	-	-	0.4	40
Mid-Range Crude Oils					
Venezuela Light	52	34	14	1.5	30
Kuwait	63	20	24	2.4	31
USA West Texas Sour	46	32	22	1.9	32
Heavy Crude Oils					
Prudhoe Bay	27	36	28	0.9	28

Saudi Heavy	60	20	15	2.1	28
Venezuela Heavy	35	53	12	2.3	24
Belridge Heavy	Non-aromatics 37%		63	1.1	-

[a] Reproduced from data in Table 1 on page 7 of test plan and pages 2-3 of the robust summary.

Category Justification

The submitter states that these naturally occurring complex mixtures follow a pattern of physicochemical, environmental fate, health, and ecological properties based on their composition so that these properties will generally correlate with the relative proportions of paraffinic, naphthenic and aromatic hydrocarbons as well as other compounds in these crude oils. Thus, the testing endpoints for the members of the Crude Oils category will be bounded by the properties of "light" and "heavy" crude oils because they bound the compositional range.

However, while the proposed grouping of the crude oils may be justifiable, it is not always clear from the data presented in the test plan how the values given by the submitter for the physicochemical and environmental fate properties correlate with differences in the compositions of paraffins, naphthenes and aromatic hydrocarbons in the crude oils.

For example, Table 1 of the test plan shows that Prudhoe Bay and Saudi Heavy crude oils have very different levels of noncyclic paraffins vs. cycloparaffins and aromatics; yet they have the same API gravity. Furthermore, only one heavy crude (Prudhoe Bay) has a relatively high aromatic number (28 %); for the lights the numbers are 19, 19, 16, and 50. These differences are important when analyzing the biodegradation of these chemicals. Saudi Light (63 % paraffins and 19 % aromatics) may be more difficult to biodegrade than Venezuela Heavy (35 % paraffins and 12 % aromatics). The submitter needs to clarify its category justification as to physicochemical properties and environmental fate.

The existing health effects data presented indicate that acute mammalian toxicity is similar for all crude oils (light or heavy) that have been tested. In addition, results of repeated-dose and developmental toxicity studies indicate that selected light and heavy crude oils share similar toxicity endpoints. However, the expectation that the mammalian toxicities of these crude oils will correlate with the PAC content of the crude oils is not supported by the data provided in the test plan. In addition, the proportion of aromatic hydrocarbons in the crude oils does not always correlate with API gravity (e.g., Lost Hills "Light" contains 50% aromatics while Venezuela "Heavy" contains 12% aromatics), even though the submitter notes that naphthenic crudes have low API gravity and a higher aromatic content than paraffinic crudes with high API gravity and are rich in straight and branched paraffins. Furthermore, from the information provided by the submitter, it is not possible to determine whether volume percent aromatic content can be an accurate indicator of 3-7 ring PAC content, which is considered by the submitter to be the basis of observed mammalian toxicity in the crude oils. The submitter notes that although few large-scale toxicity studies have been performed on crude oils, many studies are available that characterize mammalian toxicity of products derived from crude oil. The submitter should use these and any other applicable studies to demonstrate the direct correlation between mammalian toxicity and 3-7 ring PAC content.

Although adequately conducted studies were submitted for fish and aquatic invertebrates, it is not clear that adequate data exist to characterize the *extremes* of aquatic toxicity of crude oils to aquatic organisms because the substances that have been tested were not adequately characterized with respect to components that could affect their toxicity. For example, the test plan indicates on page 10 that the acute toxicity to aquatic organisms is "attributed to those water-soluble components that are either saturates (aliphatic or alicyclic) or mono-or di-aromatics." None of the substances tested for aquatic toxicity, however, were characterized with respect to these water-soluble components. The test plan also reported that crude oils contain "sulfur, oxygen and nitrogen compounds, organometallic complexes notably of sulfur and vanadium, and dissolved gases such as hydrogen sulfide." However, none of the crude oils that were tested were characterized with respect to these components, and the test plan did not indicate

whether or not the presence of these compounds could affect the aquatic toxicity of crude oils. Therefore, there is insufficient information to determine whether adequate data exist to characterize the aquatic toxicity of the Crude Oil category. Crude oils that contain the extremes of components that could affect their toxicity need to be tested before the data can be considered adequate to satisfy the SIDS endpoints.

Test Plan

Physicochemical Properties (melting point, boiling point, vapor pressure, partition coefficient and water solubility)

The data provided by the submitter for melting point, boiling point, vapor pressure, partition coefficient, and water solubility are adequate for the purposes of the HPV Challenge Program.

Environmental Fate (photodegradation, stability in water, biodegradation, fugacity)

The data provided by the submitter for photodegradation, stability in water, and biodegradation are adequate for the purposes of the HPV Challenge Program.

Fugacity. The submitter reports a summary of the distributions of 14 model hydrocarbons that were calculated using a Mackay Level I EQC fugacity model. EPA recommends that the submitter also provide estimated distributions of the model hydrocarbons based on a Level III fugacity model.

Health Effects (acute toxicity, repeated-dose toxicity, genetic toxicity, and reproductive/developmental toxicity)

The submitter expects that the selection of representative light and heavy crude oil candidates for additional testing will cover the extremes of component composition for the category. However, from data provided in Table 1, it is not apparent that the major components of these crude oils correlate with the API gravity. Therefore, EPA recommends testing crude oils that have different concentrations of known or suspected toxicants rather than non-selectively choosing a sample of heavy or light crude oil. In addition, if the submitter chooses to base the testing on extremes of PAC content, the submitter needs to better demonstrate that toxicity correlates with PAC content.

Although the percent PAC is given in Table 1 (page 7), neither the robust summaries nor the test plan provide the percentages of 3-7 ring PAC content for the tested oils to better analyze whether toxicity varies with PAC content. The submitter needs to provide more information on the percentages of 3-7 ring PAC content for the tested oils.

If appreciable amounts of metals are present in at least some of the crude oils, the proposed tests should be conducted using a range of metal content. Lacking this information, EPA reserves judgment on the additional number and types of tests that need to be conducted.

The submitted data for acute toxicity are adequate and no further testing is needed for the purposes of the HPV Challenge Program. The submitted dermal repeated-dose toxicity, genotoxicity, and developmental toxicity studies are limited because they cannot establish a true dose-response and additional testing is needed on crude oils with low and high levels of PACs pending additional information from the submitter that more clearly establishes the relationship between toxicity and PAC content. The submitter also needs to provide data for samples that contain other potentially toxic components. No adequate data were submitted for reproductive toxicity. The submitters' plan to conduct combined repeated-dose and reproductive/developmental toxicity screening studies is acceptable. However, the submitter needs to more completely justify using the dermal route of exposure and needs to choose samples that have high and low concentrations of suspected toxicants, which may affect the type of crude oil samples chosen as well as the number of tests needed. EPA has the following comments on the submitted data.

Acute Toxicity. No additional acute toxicity testing is needed for light crude oils, as adequate data are available on Beryl light crude. The submitter needs to provide a full robust summary for acute oral testing on one of the heavy crude oils (preferably Belridge Heavy since this oil has the highest amount of aromatics) to complete the documentation for this endpoint. A full robust summary should also be prepared for dermal toxicity for one of the heavy crude oils (again, Belridge Heavy would be a good choice).

Repeated-Dose Toxicity. The 13-week dermal toxicity assays on light and heavy crude oils are limited because of the failure to occlude the site of application of the test material. Results for one developmental toxicity assay indicated that the use of Elizabethan collars may not have prevented ingestion, raising uncertainty as to the dose response.

The submitter's plan to conduct a combined repeated-dose and reproductive/developmental toxicity screening study (OECD TG 422) on light and heavy crude oils is acceptable only if the samples contain different levels of known or suspected toxicants. For example, if the submitter can more clearly demonstrate that the toxicity of similar substances is correlated with PAC content, one of the oils should have a high 3-7 ring PAC content and the other one a low PAC content.

Dermal studies submitted for the acute and repeated-dose toxicity endpoints demonstrated internal systemic effects, thus indicating that the material was absorbed. However, if available, additional information on dermal absorption rates needs to be provided to assure that absorption is not limited. These additional data are needed because inhalation is also a possible exposure route and if dermal absorption is limited, then testing by the inhalation route should be considered. In addition, the summary for the developmental toxicity study on Lost Hills light crude oils raised the issue that some ingestion may have occurred despite the use of Elizabethan collars (administration sites were not occluded in this study). Therefore, the submitter needs to make sure the test substance is occluded if testing is done by the dermal route.

Genetic Toxicity. Mutagenicity testing for the category is adequate for light crude oils, based on the test for South Louisiana crude. The other mutagenicity studies were limited in the number of strains (only one strain used for several studies) as well as the use of metabolic activation as the only test condition for most studies. Given these limitations and the positive results obtained for several of the crude oil samples, a more complete mutagenicity study (e.g., OECD TG 471 using all strains both with and without metabolic activation) on a crude oil with a high PAC content is needed if a correlation between PAC content and toxicity is more clearly established.

The test plan (page 13) mentions that the DMSO extraction procedure created a test material enriched in 3-5- and 3-7 ring PAC content. If possible, the robust summaries need to provide data showing the final compositional information (percent 3-5 or 3-7 ring PAC) for the test substances.

The chromosomal aberration endpoint is satisfied by *in vitro* assays in Chinese hamster ovary cells exposed to Lost Hills light crude or Belridge heavy crude oils.

The submitter needs to remove references to Lockard *et al.* (1982) from the test plan (page 13) since the *in vitro* study was assigned a Klimisch code of 4. Also, no citation was provided for Petrilli *et al.* (1980), a bacterial mutagenicity assay on Arab light crude (page 13 of the test plan).

Reproductive Toxicity. No adequate data were submitted. The submitter's plan to conduct a combined repeated-dose toxicity and reproductive/developmental toxicity screening study (OECD TG 422) on light and heavy crude oils may be acceptable with qualifications (see additional suggestions for PAC content under repeated-dose toxicity). In addition, see comments under repeated-dose toxicity for concerns about using the dermal route for testing.

Developmental Toxicity. The dermal developmental toxicity studies on Belridge heavy and Lost Hills light crude oils do not satisfy this endpoint because ingestion of the test material may have occurred, raising uncertainty as to the accuracy of the dose response.

The submitter's proposed testing under OECD TG 422 is acceptable if, as noted earlier, the dermal route is adequately justified and the test site is occluded and the samples contain high and low concentrations of known or suspected toxicants.

Ecological Effects (fish, invertebrates, and algae)

Although the studies submitted for fish and aquatic invertebrates are adequate, it is not clear that adequate data exist to characterized the *extremes* of aquatic toxicity of crude oils to aquatic organisms because the substances that have been tested were not adequately characterized with respect to components that could affect their toxicity. Therefore, EPA reserves judgment on the fish and aquatic invertebrates toxicity endpoints pending more information on how the tested substances represent the members of the crude oils category. Also, EPA recommends testing in aquatic plants; the submitted data are not adequate because the studies were only 48 hours in duration and were conducted using an algal species that is not recommended by OECD TG 201. In addition, no chronic aquatic toxicity data were included in the test plan or robust summary document. Because the Log K_{ow} values of some components of the crude oils are >4.2, there appears to be a need for chronic aquatic toxicity data.

Some robust summaries assigned Klimisch codes of 3 (invalid) were included in the robust summary document and cited in the test plan. Studies considered to be unreliable should not be used in the test plan to support conclusions as to the toxicity of these substances. Also, values were reported in the test plan that did not have any associated robust summaries. For example, p. 22 reported daphnid EC50 values of 43 and 51 mg/L and fish LC50 values of 350 and 310 mg/L. However, these values were not reported in any of the robust summaries.

Specific Comments on the Robust Summaries

Generic comments

In several cases, robust summaries did not include the chemical composition of the different crude oils. Although some information is in the test plan, it needs to be included in each summary so that the study descriptions are complete. No summary included data on the 3-7 ring PAH composition of the tested crude oils.

Health Effects

Acute Toxicity. Robust summaries for acute oral and dermal toxicity studies on Beryl (light) crude oil did not include chemical composition data. Additional data (LD_{50}, clinical signs) within these robust summaries were provided for other kinds of crude oils. However, documentation for these oils is incomplete and needs to be expanded.

Repeated-Dose Toxicity. A robust summary for 13-week dermal toxicity assays on rats exposed to either light or heavy crude oil was missing the number of hours of daily exposure and the total skin area of application. For both oils, the summary also omitted incidence data for thyroid hypertrophy and hyperplasia (occurring at all dose levels), which are needed to determine whether there is a dose-response relationship. Study methods were equivalent to OECD TG 411 except that it is not clear whether an ophthalmological examination was conducted. The summaries also need to report NOAELs and LOAELs.

Genetic Toxicity. The first robust summary in Section 5.5 (Genetic Toxicity In Vitro), for a reverse mutation assay in *Salmonella typhimurium* strain TA98, omitted the composition of all test materials, the

number of replicates (if different from OECD guidelines), and information on cytotoxicity. The methods were similar to OECD TG 471 except that only one strain was tested and no test was conducted without exogenous S9. Slopes indicative of mutagenicity were presented for Arab light, MCSL crude and Belridge Heavy crude oils but revertant numbers were not provided. This summary needs to be expanded because it appears to be a key study, given that results for several of the samples were positive.

Robust summaries for negative chromosomal aberration assays in Chinese hamster ovary cells exposed to Lost Hills light or Belridge heavy crude oils were missing the chemical composition of the test materials (which are presented in the test plan, page 7 and Table 1).

Developmental Toxicity. Robust summaries for pre- and postnatal developmental toxicity studies of Belridge heavy and Lost Hills light crude oils in rats exposed dermally on gestational days 0-19 were missing the chemical composition of the test materials (which were presented in the test plan, page 7 and Table 1) and the specific serum chemistry parameters (if different from OECD TG 414). The summary on Belridge heavy omitted NOAEL/LOAEL fields for maternal and developmental toxicity. The summary on Lost Hills light has a typographical error in the Reproductive and Fetal Evaluations fields: the first sentences mentioning 200 mg/kg/day group probably should read “2000 mg/kg/day.” Study methods were equivalent to OECD TG 414, except that the group sizes were 12 rather than 20; however, for both test oils, a total of 24 dams were tested at the highest dose (considering both the pre- and postnatal tests).

Followup Activity

EPA requests that the submitter advise the Agency within 90 days of any modifications to its submission.